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ORIGINAL PAPER

Investigating a lotic microbial community following a severe detergent spill

Amitai Or · Uri Gophna

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Abstract A large non-ionic detergent spill affected the Yarqon stream, where water sampling was performed prior to the spill as a part of the stream's routine sampling and during and after the event. Following the spill, a large foam layer was observed for about 3–4 days accompanied by death of all fauna in the stream. Despite a large quantity of freshwater that was introduced to the stream as an emergency measure, a drastic decrease in dissolved oxygen was also observed. A rapid reduction in bacterial diversity and richness, as measured by automated ribosomal intergenic spacer analysis, was also evident, as microbial assemblages changes accompanied pollutant exposure. However, this analysis showed that the microbial assemblages of the stream were quick to recover and became similar to pre-spill communities as early as a week after the spill. These findings suggest that bacterial assemblages are much more robust to large anthropogenic disturbances than expected.

Keywords Stream · Spill · Detergents · Recovery · Bacteria

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A. Or · U. Gophna (✉)
Department of Molecular Microbiology and Biotechnology, George S. Wise Faculty of Life Sciences, Tel Aviv University, 69978 Ramat Aviv, Tel Aviv, Israel
e-mail: urigo@tauex.tau.ac.il

Introduction

Riverine ecosystems are diverse and ever-changing, and may be prone to disturbance (Stanley et al. 2010). These ecological disturbances are defined as "any relatively discrete event in time that is characterized by a frequency, intensity and severity outside a predictable range, and that disrupts ecosystem, community, or population structure and changes resources or the physical environment" (Sousa 1984). Since anthropogenic influence on the natural environment has been increasing worldwide, the issue of spills, the accidental introduction of pollutants to the natural environment, is becoming critical (Marzluff et al. 2008). An appropriate understanding of spill processes is crucial for impact assessment and environmental management (Gore et al. 1990; Aronson and Le Floc'h 1996; Munné et al. 2003; Sarrazin and Barbault 1996; Tavasi et al. 2004). Although the response of microbial assemblages to various spills, such as oil (Mendelssohn et al. 2012), pesticides (Pesce et al. 2006), herbicides (Lawrence et al. 2001), fertilizers (Peacock et al. 2001), and heavy metals (Clements et al. 2000), has been extensively documented, the impact of detergent spills on the lotic environment has not been adequately studied.

On November 6, 2008, a massive quantity of non-ionic detergents and other possible reagents (see Supplementary Table 1) reached the Kfar-Saba Hod-Hasharon wastewater treatment plant due to a large fire. This, in turn, caused a large volume of detergent, estimated at several tons, to enter the Yarqon stream (Raz 2009). Along the 20 km of detergent polluted stream, there was massive animal mortality, and 106 t of dead fish had to be disposed of. As an emergency measure, an additional 1,600 cubic meters of freshwater per hour were channeled into the stream from the Rosh-Ha'Aiyn springs for 13 days, far exceeding the stream's usual water flow, which is about 270 cubic meters per hour.

Ecological disasters are generally unexpected (Sousa 1984; Resh et al. 1988). It is thus difficult to obtain samples that reflect the status of an ecosystem before, during and after such an event. In the current study, we were fortunate to have had the opportunity to survey the Yarqon stream before the spill, using a robust, well-tested, fingerprinting method (ARISA) common in the study of marine and aquatic systems (Fisher and Triplett 1999; Zurel et al. 2011). Furthermore, our sampling locations after the spill were the same as those before (Or and Gophna 2011, 2012), which allowed us to compare both the physicochemical parameters and the alpha and beta diversity of free-living bacterial assemblages before, during and after the event. We correlated our ARISA data from before, during and after the detergent spill with the corresponding physicochemical parameters of the water. Although ARISA is unable to identify the bacterial species present, it can nevertheless provide a reliable high-resolution estimate of taxonomic diversity (Kovacs et al. 2010) and allow for the comparison with bacterial communities (i.e., beta diversity). Importantly, we had utilized ARISA successfully in the Yarqon stream before during and after the spill, which enabled us to compare the spill assemblage data to our previous findings from the same study sites.

Materials and methods

Study site

The Yarqon is a slow-flowing stream whose water mostly comes from three wastewater treatment plants (WWTPs) effluent. Based on the water quality, the Yarqon can be divided into three sections (Supplementary Fig. 1): the "natural" section, from Rosh-Ha'Ayin springs to the confluence with the Qane tributary (7.5 km) that contains fresh water; the "central" section (17.5 km),which is impacted by pollution of municipal effluents at its upper reaches, with gradual recovery downstream, contains a mixture of freshwater and WWTPs effluents; and the lowermost 4-km section (downstream to the "Seven Mills weir"), which is a partially polluted estuary that contains brackish water. The "Seven mills" weir is a physical barrier that prevents the brackish water of the estuary from mixing with upstream water with the tide, and hence has highly different biological and geochemical parameters. While conducting the study, no permits were required, no endangered or protected species were involved and no privately owned or protected location was used.

Water samples collection

All water samples were collected as described previously (Or and Gophna 2011). The locations were chosen from the estimated point where spill effluent entered the river down to the estuarine "Down Ayalon" sampling site where seawater mixes with stream water (Supplementary Fig 1). The sampling dates were from prior to the beginning of the reported event and up to the time where a return-to-normal levels of COD, BOD and non-ionic detergents levels were observed. Five different sampling rounds were made where the 10.09.08 constituted a "pre-spill" sampling round, part of a routine sampling of the stream. The following rounds of the 07.11.08, 09.11.08 and 10.11.08 represent samples taken during the spill where a visible foam layer was still present in the stream. The 16.11.08 sampling round represents a post-pollution state, 3–4 days after the foam disappeared, and the final round 24.11.08 represents a longer recovery, 2 weeks time after the spill. Two 1 mL samples of water were taken from the water at each site, and DNA was extracted from the pooled 2 mL. Due to the suddenness of the spill, only 21 samples out of the possible 30 samples (No. of time points × No. of sampling sites) were retrieved (Table 1).

Table 1 Nomenclature of the pre- and post-spill samples by location, date and cluster image color

Running Number	Location	Sampling date	Sample name
1	Hod-Hasharon Kfar-Saba WWTP	10.09.08	Pre (Black)
2	Hod-Hasharon Kfar-Saba WWTP	09.11.08	Spill_1 (Black)
3	Down Qane	10.09.08	Pre (Red)
4	Down Qane	09.11.08	Spill_1 (Red)
5	Down Qane	10.11.08	Spill_2 (red)
6	Down Qane	16.11.08	Post_1 (Red)
7	Down Qane	24.11.08	Post_2 (Red)
8	Agricultural Dam	10.09.08	Pre (Green)
9	Agricultural Dam	09.11.08	Spill_1 (Green)
10	Agricultural Dam	10.11.08	Spill_2 Green)
11	Agricultural Dam	16.11.08	Post_1 (Green)
12	Agricultural Dam	24.11.08	Post_2 (Green)
13	Seven Mills	10.09.08	Pre (Orange)
14	Seven Mills	09.11.08	Spill_1 (Orange)
15	Seven Mills	10.11.08	Spill_2 (Orange)
16	Seven Mills	16.11.08	Post_1 (Orange)
17	Seven Mills	24.11.08	Post_2 (Orange)
18	Down Ayalon	10.09.08	Spill_1 (Blue)
19	Down Ayalon	10.11.08	Spill_2 (Blue)
20	Down Ayalon	16.11.08	Post_1 (Blue)
21	Down Ayalon	24.11.08	Post_2 (Blue)

"Pre" represents 10.9.08; "Spill_1" represents 09.11.08; "Spill_2" represents 10.11.08, "Post_1" represents 16.11.08; and "Post_2" represents 24.11.08. Hod-Hasharon Kfar-Saba WWTP = black; Agricultural Dam = green; Down Qane = red; Seven Mills = orange; and Down Ayalon = blue

Environmental parameters

Several environmental parameters were measured while collecting the samples: dissolved oxygen and temperature were measured by a YSI 55 dissolved oxygen meter (YSI, Ohio USA); pH was measured by an H1 9025 pH meter (HANNA Instruments, Italy) and conductivity was measured by an H1 3733 conductivity meter (HANNA instruments, Italy). Turbidity was measured by a HACHI 21000p turbidometer. Biological oxygen demand (BOD) and chemical oxygen demand (COD) were analyzed by the USA EPA-approved standard procedures SM 5210 B and SM 5220 D, respectively, and nutrient concentrations (Total N, NO_3, NH_3-N, NO_2 and Total P) were analyzed by SM 4500-NO_3-B, SM 4500-NH_3 C, SM 4500-NO_2 B and SM 4500-P B, C colorimetric methods, respectively. The detergents were measured by SM-5550 C for Anionic (MBAS) and by a Spectroquant surfactants kit Test (Darmstadt, Germany) for non-ionic and Cationic detergents. Hydrocarbons of oil and grease were measured by SM 5220 F. All the environmental data were plotted by Microsoft Excel® software.

DNA extraction, processing and analysis

All water samples were collected and DNA was extracted as described previously (Or and Gophna 2011). Data were analyzed using the PAST software package (Hammer, 2001).

Results

Immediate effects of the spill on bacterial diversity

First, we analyzed physicochemical parameters together with the biological ARISA-based data to identify potential correlations. We noted highly elevated non-ionic detergent (Fig. 1a), COD (Fig. 1b) and BOD (Supplementary Fig 3) levels at the Kfar-Saba Hod-Hasharon WWTP sampling point on 07.11.08 (420, 2,360 and 400 mg/L, respectively).

The non-ionic detergents made their way downstream over the following 2 days, accompanied by a ten-fold drop in dissolved oxygen levels (supplementary Fig. 2). The same trend was observed at the Down Ayalon and Seven Mills sampling sites on 9.11.08 and 10.11.08. The richness and dominance indices show that the bacterial population was highly affected by the chemical changes in the water at the 07.11.08, 09.11.08 and the 10.11.08 samplings time points. Thus, we conclude that the downstream flow of detergents into the stream over 3–4 days (visible as a foam layer) likely caused a loss of bacterial diversity along the stream. The increase in dominance suggests that a few OTUs increased in abundance, possibly because of their enhanced resistance to detergents and/or ability to grow under oxygen-poor conditions.

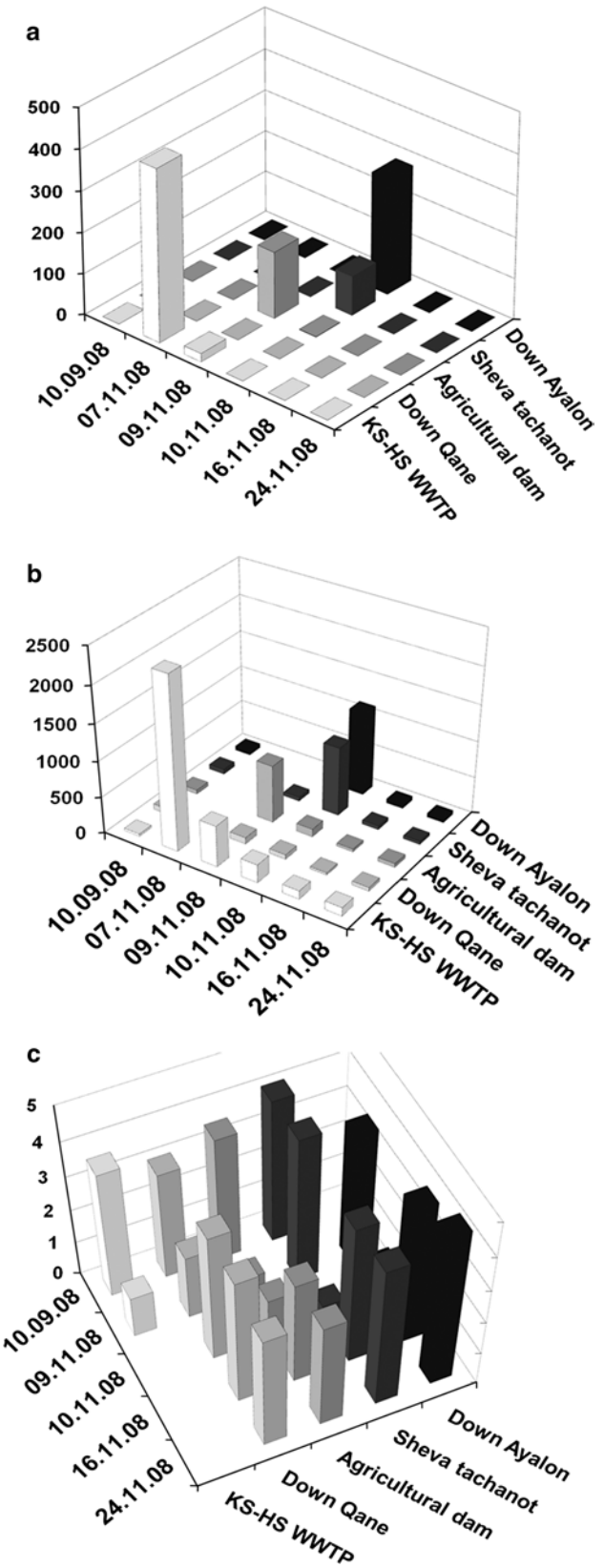

Fig. 1 Levels of: **a** non-ionic detergents; **b** COD; **c** Shannon index; in the Yarqon stream. The distribution of the measurements is displayed according to both chronological time and geographic location

Alpha and beta diversity indicate rapid recovery of bacterial assemblages

Remarkably, a return of bacterial diversity comparable to the pre-spill state, indicated by the Shanon, OTU richness and dominance indices, could be observed as early as the sampling round on 16.11.08. This return correlates with the recovery in COD and dissolved oxygen levels, which occurred, notably, only after the non-ionic detergents had been flushed out of the stream.

We subsequently examined beta diversity and performed hierarchical clustering based on the ARISA data (Fig. 2). This analysis showed a clustering pattern in which most of the "spill" time points branched together in clusters D and E, both of which were nested in a larger cluster. This clustering highlights the dramatic effect of the spill on the bacterial assemblages of the stream. In contrast, the clusters A, C and F were each made up exclusively of samples from the Seven Mills, Agricultural Dam and Down Ayalon sampling sites, respectively. Thus, these three latter clusters show a strong spatial separation effect typical of many streams (Winter et al. 2007). The B cluster grouped together samples from both pre-spill and post-spill time points, and may reflect the recovery of bacterial populations after the detergents were completely gone. When analyzing the ARISA-based microbial composition across sites and time points using Bray–Curtis similarities between samples, one can observe the speed of response of the free-living bacterial assemblages (Fig. 2). Most samples taken during the 2 days of the spill clustered together (clusters D and E), with the exception of the earliest time points in the most downstream samples at Ayalon and Seven Mills (labeled orange and blue, respectively), sites which were not exposed to the detergent. Notably, the clusters that were observed in Fig. 2 were statistically significant by ANOSIM, where each cluster (A–F) was defined as a group. The analysis confirmed that the Bray–Curtis-based clusters observed (Fig. 2) were indeed well separated ($R = 0.65$, $P < 0.05$, for pair-wise ANOSIM values; see Table 2).

In contrast, when performing ANOSIM and grouping samples by either sampling locations or sampling times, the groups were not distinct. As evident from the described results, the free-living bacterial assemblages of the stream were heavily impacted by the spill. The simultaneous rise of BOD, COD, non-ionic detergent levels and the concomitant decline of the Shannon (Fig. 1c), richness (supplementary Fig. 5) indices and DO levels highlight the immediate and dramatic response of the stream's free-living bacterial assemblages to the spill.

Freshwater ecosystems are inherently pollution-sensitive, and this is especially true for low flow streams with low dilution capacities (Smakhtin 2001). Nevertheless, the bacterial assemblages in our study quickly recovered. While this may be somewhat expected, since the detergents were gradually flushed out of the stream, the speed of recovery was nevertheless striking. In the locations where we obtained samples both before and a week or so after the spill (clusters A, B and C), pre- and post-spill samples clustered together, indicating that as early as a week after the spill, the bacterial communities were similar in composition to what they had been before that event. This strongly suggests that the stream regained its former pre-disturbance

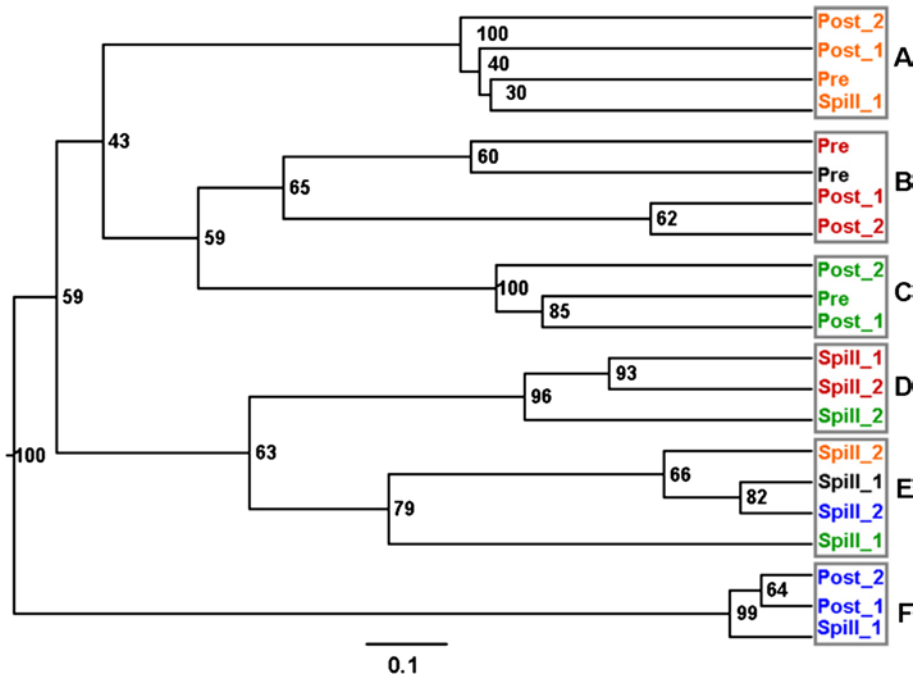

Fig. 2 A *dendrogram* representing Bray–Curtis similarity-based clustering. The bootstrap support values are displayed at the branching points as percentages. The sampling times are represented by the following headings: "*Pre*" represents 10.9.08; "*Spill_1*" represents 09.11.08; "*Spill_2*" represents 10.11.08, "*Post_1*" represents 16.11.08; and "*Post_2*" represents 24.11.08. The sampling locations are indicated by *color*: Hod-Hasharon Kfar-Saba WWTP = *black*; Agricultural Dam = *green*; Down Qane = *red*; Seven Mills = *orange*; and Down Ayalon = *blue* (color figure online)

Table 2 ANOSIM results of the Bray–Curtis clusters

	A	B	C	D	E
B	1 (0.029)*				
C	1 (0.029)*	0.87 (0.027)*			
D	1 (0.028)*	0.667 (0.029)*	1 (0.037)*		
E	0.79 (0.028)*	0.688 (0.027)*	0.667 (0.048)*	0.52 (0.044)*	
F	1 (0.028)*	1 (0.027)*	1 (0.045)*	0.675 (0.097)	0.482 (0.105)

The first number in each cell is the *R* value; the number in brackets is its corresponding *P* value. *R* values closer to zero indicate a weak separation. The Global *R* statistic for all the groups was 0.65, $P < 0.05$

* Significant result ($P < 0.05$)

bacterial characteristics, present prior to the detergent spill, and that the robust microbial community recovered rapidly, unlike the fauna (Raz 2009).

Discussion

The low levels of diversity during the spill imply that some taxa were depleted, yet no unusual proliferation of a specific taxon was observed after the spill, which could perhaps have been expected due to the elimination of certain protozoan predators (Hornák et al. 2005; Hijnen et al. 2006) or competitors (Pace and Funke 1991). The most likely explanation for this observation is that none of the bacterial taxa present was sufficiently detergent-resistant to directly benefit from the spill. The quick recovery rate we observed may be attributed either to resistant forms such as spores, dormant bacteria, and sediment submerged cells, or to a flux of planktonic bacteria from the unaffected upstream region (Or and Gophna 2011, 2012; Or et al. 2013). The latter explanation, which is perhaps the simplest, reinforces the need for preserving such natural and unpolluted segments of the stream, not exposed to treated wastewater. These clean segments are a reservoir of "naturally occurring bacteria," which may be critical for restoring stream microbial populations following disturbance.

The best-studied bacterial community recoveries following spill events involve oil spills, the most recent being the spill at Deep Water Horizon. Various aspects of this oil spill were studied (Camilli et al. 2010; Hazen et al. 2010; Kostka et al. 2011), including bacterial community structure. However, the main focus, naturally, was on the degradation of oil, a carbon source, by bacteria. The degradation of detergents has been studied in the laboratory, in systems in which water flow is almost never a part of the experimental conditions (Payne 2004; Wagener and Schink 1988; Okpokwasili and Olisa 1991; Baker et al. 1941). Nevertheless, when discussing the effects of bacterial degradation in riverine ecosystems, one should also consider the effects of water flow, which are often critical. Generally, during a river spill, there is not sufficient time to allow for the expansion of detergent degraders. Consequently, we see only the effect of detergents on bacteria rather than vice versa. Most studies that deal with the effect of detergents on the riverine environment deal exclusively with the flora and fauna, not with microorganisms (Wagener and Schink 1988). Furthermore, studies of riverine community dynamics before and after a detergent spill have not been carried out before, to the best of our knowledge, and we hope that this work will encourage future study. The microbial community serves as the scaffold of the entire ecological web by providing primary and secondary producers, decomposers, and symbionts. Yet, the severity of damage sustained by the flora and fauna may prevent them from benefiting from the services of the restored microbial community. An important question that will have be addressed by future research is whether the microbial community's ability to restore itself is in any way a positive sign for the more fragile communities of flora and, especially, fauna (Pimm 1984; Niemi et al. 1993; McCann 2000; O'Gorman and Emmerson 2009).

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